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Appendix A
U.S. Ser. No. 09/634,252
Version with Markings to Show Changes

The title of the application is amended as follows:

METALLOPROTEASE-DISINTEGRIN[S SVPH3-13 AND] ADAM23 (SVPH3-17) [DNA]NUCLEIC ACIDS

The paragraph at page 1, line 25, to page 2, line 1, is amended as follows:

Also [incluced] included are the membrane-anchored ADAMs (**A** Disintegrin **A**nd **M**etalloproteinase), which are multimeric molecules consisting of metalloproteinase, disintegrin-like, cysteine rich, and epidermal growth factor domains. *See* Black, R.A. and White, J.M., (1998) "ADAMs: focus on the protease domain," Curr Opin Cell Biol 10, 654-659 (in process); Wolfsberg, T.G. and White, J.M. (1996) "ADAMs in fertilization and development," Dev Bio 180, 389-401, all of which are herein incorporated by reference. The ADAMs family includes fertilin-α and meltrin-α, both of which are involved in membrane or cell-cell fusion. Specifically, the disintegrin domain of fertilin-α and meltrin-α have been implicated in sperm/egg fusion and myoblast fusion, respectively.

The paragraph at page 2, lines 18-27, is amended as follows:

ADAMS 1-6 have been implicated in fertilization and/or spermatogenesis (Barker, H.L., Perry, A.C., Jones, R., and Hall, L., *Biochim Biophys Acta*, 1218, 429-31, 1994; Blobel, C.P., Wolfsberg, T.G., Turck, C.W., Myles, D.G., Primakoff, P., and White, J.M., *Nature*, 356, 248-252, 1992; Evans, J.P., Schultz, R. M., and Kopf, G.S., *J. Cell Sci*, 108, 3267-3278, 1995; Perry, A.C., Barker, H.L., Jones, R., and Hall[.], L., *Biochim Biophsy Acta*, 1207, 134-137, 1994; Perry, A.C., Gichuhi, P.M., Jones, R., and Hall, L., *Biochem J.*, 307, 843-850, 1995; Perry, A. C., Jones, R., and Hall, L., *Biochem J.*, 312, 239-244, 1995; Wolfsberg, T.G., Bazan, J.F., Blobel, C.P., Mules, D. G., Primakoff, P., and White, J.M., *Proc Natl Acad Sci USA*, 90, 10783-10787, 1993; and Wolfsberg, T.G., Straight, P.D., Gerena, R.L., Huovila, A.P., Primakoff, P., Myles, D.G., and White, J. M., *Dev Biol*, 169, 378-383, 1995).

The paragraphs at page 4, line 12 through page 5, line 5 are amended as follows:

In addition, when a peptide fingerprint of an unknown protein is obtained, it can be compared to a database of known proteins to assist in the identification of the unknown protein using mass spectrometry (W.J. Henzel et al., *Proc. Natl. Acad. Sci. USA* 90:5011-5015, 1993; D. Fenyo et al., *Electrophoresis* 19:998-1005, 1998). A variety of computer software programs to facilitate these comparisons are accessible via the Internet, such as Protein Prospector ([Internet site:]prospector.uscf.edu), MultiIdent ([Internet site: www.]expasy.ch/sprot/multiident[.html]), PeptideSearch ([Internet site: www.]mann.embl-heiedelberg.de...deSearch/__FR_PeptideSearch Form[.html]), and ProFound ([Internet site: www.]chait-sgi.rockefeller.edu/cgi-bin/prot-id-frag[.html]). These programs allow the user to specify the cleavage agent and the molecular weights of the fragmented peptides within a designated tolerance. The programs compare these molecular weights to protein molecular weight information stored in databases to assist in determining the identity of the unknown protein. Accurate information concerning the number of fragmented peptides and the precise molecular weight of those peptides is required for accurate identification. Therefore, increasing the accuracy in determining the number of fragmented peptides and their molecular weight should result in enhanced likelihood of success in the identification of unknown proteins.

In addition, peptide digests of unknown proteins can be sequenced using tandem mass spectrometry (MS/MS) and the resulting sequence searched against databases (J.K. Eng, et al., *J. Am. Soc. Mass Spec.* 5:976-989 (1994); M. Mann and M. Wilm, *Anal. Chem.* 66:4390-4399 (1994); J.A. Taylor and R.S. Johnson, *Rapid Comm. Mass Spec.* 11:1067-1075 (1997)). Searching programs that can be used in this process exist on the Internet, such as Lutefisk 97 ([Internet site: www.]lsbc.com:70/Lutefisk97[.html]), and the Protein Prospector, Peptide Search and ProFound programs described above. Therefore, adding the sequence of a gene and its predicted protein sequence and peptide fragments to a sequence database can aid in the identification of unknown proteins using tandem mass spectrometry.

The paragraph at page 11, lines 18-21, is amended as follows:

SVPH3-13 and 3-17 proteinases are [a] members of the snake venom protease family. SVPH3-13 (ADAM22) polypeptide (SEQ ID NO:3) encodes a portion of the

disintegrin domain. SVPH3-13 (ADAM22) DNA (SEQ ID NO:5) encodes an SVPH3-13 (ADAM22) polypeptide (SEQ ID NO:6), which lacks a portion of the amino terminal signal sequence.

The paragraph at page 12, lines 2-4, is amended as follows:

SVPH3-13 (ADAM22) is specifically expressed in brain by Northern analysis (Example 1). SVPH3-17 (ADAM23) is specifically expressed in brain and heart by Northern analysis (Example [2]1). Therefore, SVPH3-13 and SVPH3-17 may be involved in neurogenesis.

The paragraph at page 13, lines 15-27, is amended as follows:

In a particular embodiment, the invention relates to certain isolated nucleotide sequences that are free from contaminating endogenous material. A "nucleotide sequence" refers to a polynucleotide molecule in the form of a separate fragment or as a component of a larger nucleic acid construct. The nucleic acid molecule has been derived from DNA or RNA isolated at least once in substantially pure form and in a quantity or concentration enabling identification, manipulation, and recovery of its component nucleotide sequences by standard biochemical methods (such as those outlined in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd [s]ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989)). Such sequences are preferably provided and/or constructed in the form of an open reading frame uninterrupted by internal non-translated sequences, or introns, that are typically present in eukaryotic genes. Sequences of non-translated DNA can be present 5' or 3' from an open reading frame, where the same do not interfere with manipulation or expression of the coding region.

The paragraph at page 39, lines 16-19, is amended as follows:

Similarly, all or a portion of the nucleic acids of SEQ ID 2, including oligonucleotides, can be used by those skilled in the art using well-known techniques to identify the human chromosome 2, and the specific locus 2q33, that contains the DNA of [SVPH3-13] SVPH3-17 (ADAM23) family members.

The paragraph at page 39, line 25 through page 40, line 4 is amended as follows:

For example, chromosomes can be mapped by radiation hybridization. First, PCR is performed using the Whitehead Institute/MIT Center for Genome Research Genebridge4 panel of 93 radiation hybrids ([http://www-]genome.wi.mit.edu/ftp/distribution/_human_STS_releases/july97/rhmap/genebridge4[.html]). Primers are used which lie within a putative exon of the gene of interest and which amplify a product from human genomic DNA, but do not amplify hamster genomic DNA. The results of the PCRs are converted into a data vector that is submitted to the Whitehead/MIT Radiation Mapping site on the internet ([http://www-]seq.wi.mit.edu). The data is scored and the chromosomal assignment and placement relative to known Sequence Tag Site (STS) markers on the radiation hybrid map is provided. The following web site provides additional information about radiation hybrid mapping: [http://www-]genome.wi.mit.edu/ftp/distribution/human_STS_releases/july97/_07-97.INTRO[.html]).

The paragraph at page 47 line 24 to page 48, line 3, is amended as follows:

Similarly, these reagents can be used to investigate [constituitive] constitutive and transient expression of SVPH3-13 (ADAM22) or 3-17 (ADAM23) RNA or polypeptides. SVPH3-13 (ADAM22) and 3-17 (ADAM23) DNAs can be used to determine the chromosomal location of SVPH3-13 and 3-17 DNAs and to map genes in relation to this chromosomal location. SVPH3-13 and 3-17 DNAs can also be used to examine genetic heterogeneity and heredity through the use of techniques such as genetic fingerprinting, as well as to identify risks associated with genetic disorders. SVPH3-13 and 3-17 DNAs can be further used to identify additional genes related to SVPH3-13 or 3-17 DNAs and to establish evolutionary trees based on the comparison of sequences. SVPH3-13 and 3-17 DNAs and polypeptides can be used to select for those genes or proteins that are homologous to SVPH3-13 or 3-17 DNA or polypeptides, through positive screening procedures such as Southern blotting and immunoblotting and through negative screening procedures such as subtraction.

The paragraphs at page 58, line 18 through page 59, line 5 are amended as follows:

As set forth above, a polypeptide or peptide fingerprint can be entered into or compared to a database of known proteins to assist in the identification of the unknown protein using mass spectrometry (W.J. Henzel et al., Proc. Natl. Acad. Sci. USA 90:5011-5015, 1993; D. Fenyo et al., Electrophoresis 19:998-1005, 1998). A variety of computer software programs to facilitate these comparisons are accessible via the Internet, such as Protein Prospector ([Internet site:]prospector.uscf.edu), MultiIdent ([Internet site: www.]expasy.ch/sprot/multiident[.html]), PeptideSearch ([Internet site:www.]mann.embl-heiedelberg.de...deSearch/____FR_PeptideSearch Form[.html]), and ProFound ([Internet site:www.]chait-sgi.rockefeller.edu/cgi-bin/ prot-id-frag[.html]). These programs allow the user to specify the cleavage agent and the molecular weights of the fragmented peptides within a designated tolerance. The programs compare observed molecular weights to predicted peptide molecular weights derived from sequence databases to assist in determining the identity of the unknown protein.

In addition, a polypeptide or peptide digest can be sequenced using tandem mass spectrometry (MS/MS) and the resulting sequence searched against databases (J.K. Eng, et al., J. Am. Soc. Mass Spec. 5:976-989 (1994); M. Mann and M. Wilm, Anal. Chem. 66:4390-4399 (1994); J.A. Taylor and R.S. Johnson, Rapid Comm. Mass Spec.11:1067-1075 (1997)). Searching programs that can be used in this process exist on the Internet, such as Lutefisk 97 ([Internet site: www.]sbc.com:70/Lutefisk97[.html]), and the Protein Prospector, Peptide Search and ProFound programs described above.

The paragraph at page 59, lines 20-30, is amended as follows:

These antigenic determinants or epitopes can be either linear or conformational (discontinuous). Linear epitopes are composed of a single section of amino acids of the polypeptide, while conformational or discontinuous epitopes are composed of amino acids sections from different regions of the polypeptide chain that are brought into close proximity upon protein folding (C. A. Janeway, Jr. and P. Travers, *Immuno Biology* 3:9 (Garland Publishing Inc., 2nd ed. 1996)). Because folded proteins have complex surfaces, the number of epitopes available is quite numerous; however, due to the conformation of the protein and steric [hinderances] hindrances, the number of antibodies that actually bind to the epitopes is less than the

number of available epitopes (C. A. Janeway, Jr. and P. Travers, *Immuno Biology* 2:14 (Garland Publishing Inc., 2nd ed. 1996)). Epitopes may be identified by any of the methods known in the art.

The paragraph at page 64, lines 15-18, is amended as follows:

Chromosomal mapping of [of] SVPH3-13 and SVPH3-17 was performed by radiation-hybrid mapping (Walter *et al.*, 1994). The GeneBridge 4 radiation-hybrid mapping panel (Research Genetics, Huntsville, AL) was screened with specific primer pairs for SVPH3-13 (ADAM22) and SVPH3-17 (ADAM23).

Claims 15 and 22-23 are amended as follows:

15 (amended). An isolated nucleic acid molecule selected from the group consisting of:

(a) a nucleic acid molecule having the sequence of[SEQ ID NO:1 or] SEQ ID NO:2;

(b) a nucleic acid molecule encoding an amino acid sequence comprising the sequence of[SEQ ID NO:3 or] SEQ ID NO:4;

[(c) a nucleic acid molecule that hybridizes to either strand of a denatured, double-stranded DNA comprising the nucleic acid sequence of (a) or (b) under conditions of moderate stringency in 50% formamide and 6XSSC, at 42°C with washing conditions of 60°C, 0.5XSSC, 0.1% SDS and that is at least 90% identical to the nucleic acid sequence of (a) or (b);]

[(d) a nucleic acid molecule having the sequence of SEQ ID NO:5;]

[(e) a nucleic acid molecule encoding an amino acid sequence comprising the sequence of SEQ ID NO:4 or SEQ ID NO:6;]

([f]c) a nucleic acid molecule that encodes a fragment of the polypeptide of SEQ ID NO:4 [or SEQ ID NO:6]having disintegrin[or proteinase] activity;

[(g) a nucleic acid molecule encoding an amino acid sequence comprising amino acids 1-697 of SEQ ID NO:6, amino acids 698-721 of SEQ ID NO:6,

amino acids 722-867 of SEQ ID NO:6; amino acids 400-499 of SEQ ID NO:6, or amino acids 500-697 of SEQ ID NO:6; and]

([h]d) a nucleic acid molecule encoding a fragment of the amino acid sequence of SEQ ID NO:4 having disintegrin activity and [an amino acid sequence]comprising amino acids[1-58 of SEQ ID NO:4; amino acids 59-286 of SEQ ID NO:4; amino acids 287-495 of SEQ ID NO:4; amino acids] 496-599 of SEQ ID NO:4[; amino acids 600-786 of SEQ ID NO:4; amino acids 787-817 of SEQ ID NO:4; or amino acids 818-832 of SEQ ID NO:4]; and

(e) a nucleic acid molecule encoding a fragment of the amino acid sequence of SEQ ID NO:4 having disintegrin activity and comprising an amino acid sequence selected from the group consisting of amino acids 499 through 530 of SEQ ID NO:4 and amino acids 532 through 586 of SEQ ID NO:4.

22 (amended). A method for the [production]expression of an[SVPH3-13 or] SVPH3-17 (ADAM23) disintegrin polypeptide comprising culturing a host cell of claim 21 under conditions promoting expression of the vector of claim 21.

23 (amended). The method of claim 22, further comprising recovering the expressed polypeptide[produced].